

## Phytoextraction of Lead from Pb Contaminated Soil by *Brassica juncea* as Affected by Chelating Agents

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### Abstract

A screen house experiment was conducted to study the effect of chelating agents on phytoextraction of Pb using nitrilotriacetic acid (NTA), diethylenetriaminepentaacetic acid (DTPA), cyclohexanediaminetetraacetic acid (CDTA) and citric acid (CA) (at 3 mmol/kg soil) and farmyard manure (FYM) (at 3%) by *B. juncea* in the sewage sludge unamended and amended (at 3%) soil. Dry matter yield of roots and shoots of *B. juncea* increased due to application of NTA, citric acid and FYM whereas reverse trend was observed in CDTA and DTPA treated soils as compared to control (Pb<sub>180</sub>). Application of NTA increased dry matter yield of shoots by 51.13%. Addition of sewage sludge (at 3%) on dry weight basis was found to be beneficial in improving the plant growth. Chelating agents enhanced the Pb uptake by both roots and shoots and significantly higher values of Pb uptake by roots and shoots were observed from amended as compared to sewage sludge unamended soil. Application of NTA was found to be more effective in enhancing the Pb uptake by *B. juncea* roots and shoots than any other chelating agents at both the growth stages.

**Key words :** Phytoextraction, Lead, Pb contaminated soil, *Brassica juncea*, Chelating agents.

Continuous use of untreated effluents of mixed sewer water may lead to the accumulation of toxic elements in soils to such an amount that may prove toxic to plants. Phytoremediation of heavy metal contaminated soils is an approach with a motive to extract excessive metals from marginally heavy metal contaminated soils through plant uptake. Amongst the commercial crops grown in this region *Brassica* species has been reported to produce high biomass and accumulate significant amount of heavy metals in their tissues when induced through the addition of chelating agents (1). Use of sewage sludge and FYM as source of organic matter is a common practice. Organic matter applied through sludge has been reported to influence the bioavailability by complexation of heavy metals (2). Additions of chelating agents have been reported to increase Pb extractability in soils (3).

### Methods

The bulk surface sample of a sandy loam soil was collected from the research area of the Dry Land

Agriculture Section of CCS Haryana Agricultural University, Hisar, Haryana (India). The bulk sewage sludge sample was collected from the Sewer Treatment Plant, Industrial Estate, Okhla, New Delhi. The bulk sample of well decomposed farmyard manure (FYM) was taken from the manure pit of Dairy Farm, CCS Haryana Agricultural University, Hisar. The physico-chemical properties of soil, sewage sludge and FYM used are given in Table 1. Standard methods were followed to analyse physico-chemical properties of the soil, sewage sludge and FYM. The processed soils were divided into two portions. The first part treated with Pb using PbNO<sub>3</sub> (at 180 mg/kg) soil and second portion treated with Pb (at 180 mg/kg) and sewage sludge (at 3%). The experiment was carried out in a completely randomized design, arranged in a (2 × 6 × 6 = 72) factorial, two soil (sewage sludge unamended and amended); six amendment (control, four chelating agents and FYM) and six replications out of which three replications harvested at 50 days after sowing (DAS) and remaining three replications at 80 DAS. Ten healthy seeds of the *B. juncea* cultivar were sown in each pot in third week of October,

**Table 1.** Physico-chemical characteristics of the experimental soil, sewage sludge and FYM.

| Properties   | Soil       | Content<br>Sewage sludge | FYM    |
|--|------------|--------------------------|--------|
| Mechanical composition   |            |                          |        |
| Sand (%)   | 69.70      | -                        | -      |
| Silt (%)   | 16.50      | -                        | -      |
| Clay (%)   | 13.80      | -                        | -      |
| Textural class   | Sandy loam | -                        | -      |
| pH (1:2)   | 8.10       | 7.20                     | -      |
| EC <sub>1:2</sub> (dS/m)   | 0.50       | 2.10                     | -      |
| Organic carbon (g/kg)  | 3.20       | 122.00                   | 278.00 |
| CEC [ Cmol (P <sup>+</sup> )/kg]   | 11.80      | -                        | -      |
| CaCO <sub>3</sub> (g/kg)   | 4.00       | 2.50                     | -      |
| Available nutrients in soil (kg/ha) and total nutrients in sewage sludge and FYM (%) |            |                          |        |
| Nitrogen   | 186.40     | 1.29                     | 1.18   |
| Phosphorus   | 18.10      | 0.41                     | 0.70   |
| Potassium  | 198.50     | 0.73                     | 2.50   |
| C/N ratio  | -          | 9.46                     | 23.55  |
| Total metals (mg/kg)   |            |                          |        |
| Pb   | 11.37      | 64.20                    | 10.90  |
| Cd   | 3.22       | 7.20                     | 0.60   |
| Ni   | 11.37      | 64.20                    | 10.90  |

2005. After emergence of seedlings, only four plants per pot were allowed to grow. Four chelating agents namely, CDTA, citric acid, DTPA and NTA were applied (at 3 mmol kg/soil, 1 mmol daily for 3 days in 3 split doses) 40 DAS. The FYM (at 3%) was uniformly mixed in portion of Pb spiked and Pb spiked sewage sludge treated soil 10 weeks before sowing. Ten days after application of chelating agents half of the mustard crop (i.e. 3 replications out of 6) were harvested and remaining 3 replications of each treatment were harvested 80 DAS. To determine Pb in root and shoot sample, wet digestion was carried out in a diacid mixture of nitric acid and perchloric acid in 4 : 1 ratio. Lead in the digest was determined by using atomic absorption spectrophotometer.

## Results and Discussion

### Dry Matter Yield of Roots and Shoots

Effect of different treatments on dry matter yield of roots and shoots of *B. juncea* is shown in Table 2. The mean dry matter yield of roots was maximum, 3.29 g/pot, in Pb<sub>180</sub> + NTA and the least, 1.22 g/pot, in Pb<sub>180</sub> + DTPA treatment. The mean dry matter yield of roots was 1.18 and 2.03 g/pot in sewage sludge un-

amended and amended soils at first stage of growth, respectively and similar trend was observed at second stage also. Highest dry matter yield of roots was observed in NTA treated soils and the increase was 106.74 and 146.15% at first and second stage in sewage sludge unamended whereas the corresponding values for amended soil are 153.95 and 88.09% as compared to control (Pb<sub>180</sub>).

The mean dry matter yield of shoots ranged from 9.02 to 15.30 g/pot, the maximum in Pb<sub>180</sub> + NTA and the minimum in Pb<sub>180</sub> + DTPA treatment. The mean dry matter yield of shoots was 5.00 and 5.19 g/pot in sewage sludge unamended and amended soils at first stage of growth whereas it was 15.88 and 19.70 g/pot at second stages of growth, respectively. Application of NTA increased the dry matter yield of *B. juncea* shoots by 118.91 at first and 17.30% at second stage of growth in sewage sludge unamended soil whereas the corresponding values were 135.89 and 46.28% for amended soil as compared to control (Pb<sub>180</sub>). Similar results were also reported by Mishra (4) and Garrido et al. (5).

### Lead Uptake by Root and Shoot

There was a considerable variability among dif-

**Table 2.** Effect of chelating agents and sewage sludge on dry matter yield (g/pot) of roots and shoots of *B. juncea* crop in Pb contaminated soil.

| Treatment                | 50 DAS                      |                             | 80 DAS                                  |                  | Mean  |
|--------------------------|-----------------------------|-----------------------------|---|------------------|-------|
|                          | Without SS                  | With SS                     | Without SS                              | With SS          |       |
| <b>Root</b>              |                             |                             |   |                  |       |
| Pb <sub>180</sub>        | 0.89                        | 1.39                        | 1.56                                    | 2.10             | 1.48  |
| Pb <sub>180</sub> + CDTA | 0.79                        | 1.10                        | 1.31                                    | 2.00             | 1.30  |
| Pb <sub>180</sub> + CA   | 1.82                        | 3.21                        | 3.50                                    | 3.80             | 3.08  |
| Pb <sub>180</sub> + DTPA | 0.72                        | 1.10                        | 1.23                                    | 1.84             | 1.22  |
| Pb <sub>180</sub> + NTA  | 1.84                        | 3.53                        | 3.84                                    | 3.95             | 3.29  |
| Pb <sub>180</sub> + FYM  | 1.00                        | 1.85                        | 2.35                                    | 2.21             | 1.85  |
| Mean                     | 1.18                        | 2.03                        | 2.30                                    | 2.65             |       |
| CD (0.05)                | Soil = 0.09<br>S × T = 0.12 | Time = 0.09<br>S × C = 0.21 | Chelating agents = 0.15<br>T × C = 0.21 | S × T × C = 0.30 |       |
| <b>Shoot</b>             |                             |                             |   |                  |       |
| Pb <sub>180</sub>        | 3.70                        | 3.90                        | 15.60                                   | 17.50            | 10.17 |
| Pb <sub>180</sub> + CDTA | 3.30                        | 4.43                        | 14.60                                   | 16.20            | 9.63  |
| Pb <sub>180</sub> + CA   | 7.40                        | 5.70                        | 16.70                                   | 22.20            | 13.00 |
| Pb <sub>180</sub> + DTPA | 3.20                        | 3.90                        | 13.90                                   | 15.10            | 9.02  |
| Pb <sub>180</sub> + NTA  | 8.10                        | 9.20                        | 18.30                                   | 25.60            | 15.30 |
| Pb <sub>180</sub> + FYM  | 4.30                        | 4.00                        | 16.20                                   | 21.60            | 11.52 |
| Mean                     | 5.00                        | 5.19                        | 15.88                                   | 19.70            |       |
| CD (0.05)                | Soil = 0.64<br>S × T = 0.90 | Time = 0.64<br>S × C = 1.56 | Chelating agents = 1.10<br>T × C = 1.56 | S × T × C = 2.20 |       |

ferent types of chelating agents with regard to their effect on Pb uptake by roots and shoots of *B. juncea* (Table 3). Application of NTA increased the mean Pb uptake by roots by 225.81% as compared to Pb<sub>180</sub> alone. The mean Pb uptake by roots was 110.99 and 217.81 µg/pot in sewage sludge unamended and

**Table 3.** Effect of chelating agents and sewage sludge on Pb uptake (µg/pot) by roots and shoots of *B. juncea* crop in Pb contaminated soil.

| Treatment                | 50 DAS                        |                                | 80 DAS                                     |                    | Mean    |
|--------------------------|-------------------------------|--------------------------------|--|--------------------|---------|
|                          | Without SS                    | With SS                        | Without SS                                 | With SS            |         |
| <b>Root</b>              |                               |                                |  |                    |         |
| Pb <sub>180</sub>        | 62.41                         | 117.99                         | 216.67                                     | 257.01             | 163.52  |
| Pb <sub>180</sub> + CDTA | 64.74                         | 102.07                         | 299.29                                     | 330.29             | 199.06  |
| Pb <sub>180</sub> + CA   | 199.60                        | 386.29                         | 671.03                                     | 685.07             | 485.50  |
| Pb <sub>180</sub> + DTPA | 50.51                         | 98.03                          | 207.05                                     | 294.06             | 162.41  |
| Pb <sub>180</sub> + NTA  | 210.36                        | 442.45                         | 732.28                                     | 746.05             | 532.78  |
| Pb <sub>180</sub> + FYM  | 78.32                         | 160.03                         | 331.87                                     | 385.84             | 239.01  |
| Mean                     | 110.99                        | 217.81                         | 408.67                                     | 449.72             |         |
| CD (0.05)                | Soil = 14.82<br>S × T = 20.96 | Time = 14.82<br>S × C = 36.31  | Chelating agents = 25.67<br>T × C = 36.31  | S × T × C = 51.35  |         |
| <b>Shoot</b>             |                               |                                |  |                    |         |
| Pb <sub>180</sub>        | 148.46                        | 184.55                         | 912.27                                     | 1150.75            | 599.01  |
| Pb <sub>180</sub> + CDTA | 202.51                        | 304.22                         | 1156.86                                    | 1368.71            | 758.07  |
| Pb <sub>180</sub> + CA   | 507.09                        | 429.62                         | 1408.61                                    | 1995.20            | 1085.13 |
| Pb <sub>180</sub> + DTPA | 178.87                        | 236.38                         | 975.27                                     | 1131.85            | 630.59  |
| Pb <sub>180</sub> + NTA  | 604.79                        | 747.88                         | 1593.35                                    | 2362.25            | 1327.07 |
| Pb <sub>180</sub> + FYM  | 195.19                        | 209.09                         | 962.54                                     | 1402.33            | 692.29  |
| Mean                     | 306.15                        | 351.96                         | 1168.15                                    | 1568.52            |         |
| CD (0.05)                | Soil = 56.07<br>S × T = 79.30 | Time = 56.07<br>S × C = 137.35 | Chelating agents = 97.12<br>T × C = 137.35 | S × T × C = 194.24 |         |

amended soils at first stage of growth, respectively. Similar trend was observed at second stage of growth. Application of NTA increased the Pb uptake by *B. juncea* roots of plant grown in sewage sludge unamended and amended soils at first stage of growth was 237.06 and 275.00% but the values for roots at second stage are 237.97 and 190.28%, respectively as compared to control ( $Pb_{180}$ ). NTA was found to be more effective in enhancing the Pb uptake by roots as than other chelating agents.

The mean Pb uptake by shoots was 306.15 and 351.96  $\mu\text{g}/\text{pot}$  from sewage sludge unamended and amended soil at first stage of growth whereas the corresponding values were 1,168.15 and 1,568.52  $\mu\text{g}/\text{pot}$  at second stage of growth, respectively. Application of NTA increased the Pb uptake by *B. juncea* shoots by 307.37 and 305.24% in sewage sludge unamended and amended soils at first stage of growth but the corresponding values were 74.66 and 105.28% at second stage of growth, respectively as compared control ( $Pb_{180}$ ). The results indicate that addition of sewage sludge increased the Pb uptake by roots and shoots of *B. juncea*. The increased uptake of Pb by plants grown on NTA applied soils and other chelating agents may be ascribed to its increased availability in the effective root zone and of course higher dry matter yield of plant components (6, 7).

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